

ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum 383

Adrenergic,
cholecystokinetic and
morphine-induced effects on
extra-hepatic biliary motility

By

Carl G.A. Persson

LUND 1972



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From AB Draco*, Research and Development Laboratories,
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In this survey the following papers will be discussed.

- I. *Adrenoceptors in the cat choledochoduodenal junction studied in situ.* G. Liedberg and C.G.A. Persson. Br. J. Pharmacol. 39, 619—626, 1970.
- II. *Adrenoceptor functions in the cat choledochoduodenal junction in vitro.* C.G.A. Persson. Br. J. Pharmacol. 42, 447—461, 1971.
- III. *Excitatory effect of tetrodotoxin on an isolated smooth muscle organ.* C.G.A. Persson. J. Pharm. Pharmacol. 23, 986—987, 1971.
- IV. *The action of morphine on the cat choledochoduodenal tract.* C.G.A. Persson. Acta pharmacol. (Kbh), 30, 321—329, 1971.
- V. *Resistance to flow through the pancreatic duct by the isolated cat sphincter of Oddi.* C.G.A. Persson. Experientia, 28, 276—278, 1972.
- VI. *Adrenoceptors in the gallbladder.* C.G.A. Persson. Acta pharmacol. (Kbh), 31, 177—188, 1972.
- VII. *Effect of morphine, cholecystokinin and sympathomimetics on the sphincter of Oddi and intramural pressure in cat duodenum.* C.G.A. Persson and M. Ekman. Scand. J. Gastroent. 7, 345—351, 1972.
- VIII. *Effect of cholecystokinin on the level of cyclic AMP and on mechanical activity in the isolated sphincter of Oddi.* K.E. Andersson, R. Andersson, P. Hedner and C.G.A. Persson, Life Sci. 11, 723—732, 1972.
- IX. *Dual effects on the sphincter of Oddi and gallbladder induced by stimulation of the right great splanchnic nerve.* C.G.A. Persson. Acta Physiol. Scand. In press.

The papers will be referred to in the text by their roman numerals.

Introduction

A. Historical notes

Bergh (1942) and Dowdy Jr (1969) have reviewed the early history of our knowledge of the gallbladder and bile ducts. In 1543, Vesalius demonstrated that the common bile duct entered the duodenum, not the stomach, as had been believed previously. A muscular sphincter at the termination of the common bile duct was described by Glisson (1681), and Vater (1720) reported that the mucosa was elevated at the orifice in the duodenum. The elevation is known as the papilla of Vater (Fig. 1). The choledochoduodenal sphincter in the cat was first described by Gage (1879). Oddi (1887) re-discovered 'Glisson's sphincter' in several species and measured the resistance of the sphincter. It has since borne the name, sphincter of Oddi. Careful anatomical investigations by Boyden and associates (Eichhorn and

Fig 1

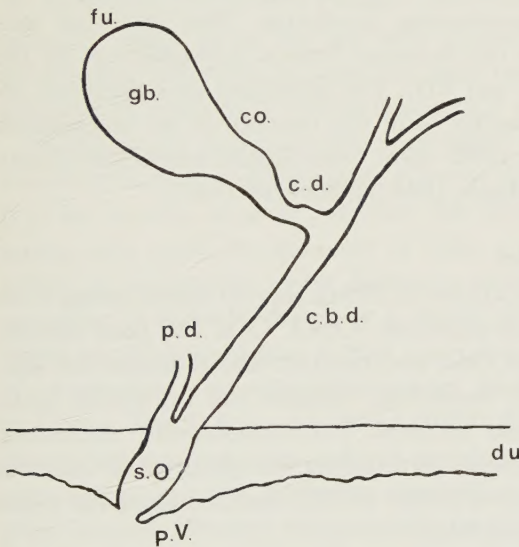


Fig. 1

Schematic drawing of the anatomy of the extra-hepatic biliary tract.

fu=fundus; gb=gallbladder; co=collum; cd=cystic duct; cbd=common bile duct; pd=pancreatic duct; sO=sphincter of Oddi; pV=papilla of Vater; du=duodenal wall

Boyden, 1955, Boyden 1957 a, Boyden 1957 b) have revealed that the sphincter of Oddi is composed of muscular tissue that differs from duodenal muscle in structure and embryological development.

Along with the increasing anatomical knowledge, studies concerning the physiology and pharmacology of the biliary tract proceeded rapidly. In 1934, Ivy reviewed over 500 papers on this subject. Current views on biliary function and motility were presented by Hallenbeck (1967).

B. Anatomical structures of importance in biliary motility

The gallbladder and the sphincter of Oddi are the most important structures in biliary dynamics (Fig. 1). Neither in the Lütken's sphincter in the collum part of the gallbladder nor in the valved cystic duct, the valves of Heister, has a true sphincteric function been established (Rothman 1965). However, experimental data showing pressure differences between the gallbladder and the common bile duct may suggest that these parts of the biliary tract have a physiological sphincter function (Benzi and Frigo 1963, Doyle and Farrar 1969). There are also indications that the common bile duct can exhibit peristaltic movements and may thus play a role in biliary motility (Daniels, Mc Glone, Job and Sawyer 1961, Hauge and Mark 1965, Ludwick 1966, Watts and Dunphy 1966, Golenhofen 1971).

It is generally agreed that the sphincter of Oddi can be influenced by the state of contraction of the surrounding duodenum. This influence may manifest itself especially when the duodenal tone is increased e.g. by the action of morphine (papers IV and VII). The possibility of a duodenal influence does not necessarily conflict with the concept of an independent sphincter mechanism operating under most physiological conditions (Lueth 1931, Bergh and Layne 1940, Magee 1946, Hallenbeck 1967).

C. Regulation of biliary dynamics

The most important mechanical effects of biliary smooth muscle seem to be produced by the hormone cholecystokinin (CCK). CCK has been demonstrated to relax the sphincter of Oddi and contract and evacuate the gallbladder (Ivy and Oldberg 1928, Sandblom, Voegtlin and Ivy 1935). Little is known about the mechanisms involved. Furthermore, the mechanical effect of CCK on the duodenal tissue surrounding the sphincter of Oddi has not been settled. The mechanism of action of CCK and the duodenal effect are discussed in papers II, VII and VIII.

The role of the autonomic nervous system in biliary dynamics has not been established. In papers I, II, V, VI and IX, special attention has been devoted to the possible role of the adrenergic nervous system in the control of biliary

motility. In the evaluation of adrenergic effects it should be of value to know the distribution and functions of adrenoceptors in biliary smooth muscle. The presence of adrenoceptors in gallbladder smooth muscle has not been established previously. In the isolated sphincter of Oddi the α -receptor has been described but there is still some doubt about the existence of a β -receptor (Crema and Berté 1963). Concerning the α -receptor function and the effect of adrenalin and noradrenalin there seems to be no agreement between *in vitro* and *in situ* findings (Crema and Berté 1963, Benzi, Berté, Crema and Frigo 1964). The adrenoceptor functions in the gallbladder and the sphincter of Oddi are demonstrated in papers I, II and VI, where also *in vitro* findings are correlated with results obtained *in situ*. In addition, effects on the biliary tract induced by the stimulation *in situ* of the adrenergic nerve supply are reported in paper IX. The nervous effects are explained in terms of adrenoceptor distribution and function, and are correlated to *in vitro* effects.

It is generally agreed that the sphincter of Oddi can function autonomously and can exhibit spontaneous activity (Bergh 1942, Magee 1946, Crema and Berté 1963, Wyatt 1967). It is not known whether the sphincter activity is myogenic or nervous in origin. Furthermore, it is controversial whether the sphincter activity promotes or inhibits the flow of bile into the duodenum (Watts and Dunphy 1966, Toouli and Watts 1972). Another question, which so far has not been studied, is how the sphincter of Oddi affects flow resistance through the main pancreatic duct. These aspects of sphincter function were studied in papers II and V.

Morphine has important effects on biliary dynamics. It increases the intraductal pressure by a spasmogenic effect at the choledochoduodenal junction. It is not known, however, whether this action is due to effects on the intestine only or on the sphincter of Oddi as well (Hallenbeck 1967, Daniel 1968). It is currently under discussion whether the spasmogenic effect of morphine is nerve-mediated or a direct effect on smooth muscle (Daniel 1968). A third possibility is that morphine acts indirectly through release of a spasmogenic non-nervous agent (cf. Burks and Long 1967). The finding by Crema, Benzi, Frigo and Berté (1965) that the morphine-spasm was not blocked by agents known to block peripheral effects of the autonomic nervous system, plus the surprising observation by the same authors that morphine was less effective intraarterially than intravenously at the choledochoduodenal junction, seemed to need confirmation as was pointed out by Daniel (1968). The effects of morphine on choledochoduodenal tissues were studied in papers IV and VII.

D. Aims of the present investigation

To summarize, the aims of the present investigation were:

1. To study the mechanism of action of CCK on biliary smooth muscle and the effect of CCK on the part of duodenum close to the sphincter of Oddi.
2. To study adrenergic effects on biliary motility.
3. To study the nature and function of the spontaneous activity of the sphincter of Oddi.
4. To study the effect of morphine on the choledochoduodenal junction.

Experimental Approach

A. Animal

In the cat, the anatomy of the biliary tract has been thoroughly demonstrated by Boyden (1957 a), who also showed that the cat sphincter of Oddi had important resemblances to man, e.g. in both species the main pancreatic duct opens into the duodenum through the sphincter of Oddi. The innervation of the cat biliary tract was described by Hirt (1934). The adrenergic nerves were further surveyed by use of the fluorescence method of Falck and Hillarp (Baumgarten and Lange 1969). The cat has been used in a number of investigations dealing with the pharmacology and physiology of biliary dynamics (e.g. Ivy 1934, Granser, Hertting, Rissel and Wewalka 1956, Benzi, Berté, Crema and Frigo 1964, Pallin and Skoglund 1964, Hallenbeck 1967). This existent knowledge of the cat biliary tract contributed to the choice of this animal for the various investigations presented in papers I—IX.

B. Methods

The various methods used for recording the activity of the gallbladder, the sphincter of Oddi and the duodenum are described in detail in the special papers. Conventional techniques were applied for measuring isometric tension changes in the isolated smooth muscle tissues (papers II, III, IV, V, VI and VIII). Intraluminal pressure changes in the gallbladder and the duodenum were measured through saline-filled open-tip catheter (papers I, IV, VI, VII and IX). In order to obtain sensitive continuous recordings of the activity and the flow resistance of the sphincter of Oddi a perfusion method was developed. The isolated sphincter (papers II, III, IV and V) and the sphincter of Oddi *in situ* (papers (I), VII and IX) were perfused at a low constant rate (3 ml/h or less) and the perfusion pressure was recorded continuously. These perfusion rates were considerably lower than those used earlier in similar *in vivo* experiments (e.g. Hedner and Rorsman 1969, Liedberg and Halabi 1970). The rates were likewise lower than those applied when the resistance to flow through the sphincter of Oddi (isolated or *in situ*) was measured using a drop counter (e.g. Benzi, Berté, Crema and Frigo 1964, Hedner and Rorsman 1969). A low perfusion rate was suggested to be of importance in achieving minimum duodenal influence on the sphincter activity recordings (paper IX), and it seemed to be of special importance

in the study of sympathetic and sympathomimetic effects on the sphincter of Oddi (see discussion in paper IX).

The recordings obtained with the open-tip catheter in the duodenal lumen were considered insufficient for the measurement of intestinal activity close to the sphincter of Oddi. Therefore a new method was developed (paper VII). Pieces of the femoral veins were taken and inserted through the duodenal wall around the sphincter of Oddi. The venous insertions were perfused in a similar manner to the sphincter. The perfusion pressure reflected sensitively and selectively the intramural duodenal pressure close to the sphincter of Oddi (papers VII and IX).

Hormonal regulation of biliary motility

During interdigestive periods the continuously secreted liver bile enters the gallbladder. The resistance of the sphincter of Oddi is of significant aid to this process (Hallenbeck 1967). It is not definitely established whether any nervous or hormonal mechanism is of importance during this gallbladder filling phase. As will be discussed below the results obtained in paper IX suggest that the activity of the adrenergic nervous system promotes the entry of bile into the gallbladder.

The mechanism of evacuation of the gallbladder can be explained as being due to hormonal effects. Boyden (1923) showed that the gallbladder emptied in response to food (fat) intake. Whitaker (1926) demonstrated that the denervated gallbladder would also evacuate when food was ingested. Ivy and Oldberg (1928) made an extract from upper intestinal mucosa which, upon i.v. injection, brought about the contraction and emptying of the gallbladder. These authors named the extracted hormone cholecystokinin. Sandblom, Voegtlin and Ivy then (1935) made the observation that CCK relaxed the sphincter of Oddi. These dual actions of CCK have been confirmed many times in different species (reviewed by Grossman 1950, Hallenbeck 1967, Jorpes and Mutt 1969). In the present work it was found that CCK consistently relaxed the sphincter of Oddi and contracted the gallbladder (papers I, II, V, VII, VIII and IX). Recently (K.E. Andersson, R. Andersson, Hedner and C.G.A. Persson 1972) it was demonstrated that prostaglandin E_2 (PGE_2) shared with CCK the dual effects on biliary muscle. Further investigations are, however, required before possible roles of PG:s in biliary dynamics can be discussed.

CCK has also been shown to have contractile effects on the intestinal muscle (Dahlgren 1967, Hedner, H. Persson, Rorsman 1967, Jorpes and Mutt 1969, Ramirez and Farrar 1970). Of special interest in biliary dynamics is the effect of CCK on the duodenal tissue that surrounds the sphincter of Oddi. Using the method with perfused venous insertions (paper VII), actions on this part of the duodenum could be selectively recorded. In these studies, CCK was shown to relax the duodenal tissue close to the sphincter of Oddi. This relaxation, recorded as decreased intramural pressure in the duodenum, occurred simultaneously with the relaxation of the sphincter of Oddi. A duodenal influence on the sphincter concomitant with the latter effect might then be defined as a facilitation of the sphincter

relaxation. Earlier, a number of reports had shown a biphasic response to CCK including a contraction of duodenal tissue (reviewed by Dahlgren 1967, Jorpes and Mutt 1969). Species differences may have contributed to these conflicting results (cf. Bertaccini and Agosti 1971) but methodological differences may also be of importance. If the method does not record selectively the activity of the part of duodenum that surrounds the sphincter of Oddi, the activity of more distal parts of the intestine may influence the recording. Actions of CCK on intestinal tissue distal to the sphincter of Oddi seem well defined as contraction and increased peristaltic activity (Hedner, H. Persson and Rorsman 1967, Ramirez and Farrar 1970, Frigo, Torsoli, Lecchini, Falaschi and Crema 1972). In papers II and VII it was confirmed that CCK can contract intestinal smooth muscle distal to the choledochoduodenal junction.

Mechanism of action of cholecystokinin

Recent investigations have suggested a probable mechanism involved in the actions of CCK. CCK seems to act directly on the smooth muscle cells in the biliary tract since its effects are not mediated by the cholinergic or the adrenergic nervous systems. This has been shown both for the gallbladder by Ivy and Oldberg 1928, Hedner 1970 and in paper IX, and for the sphincter of Oddi in the present studies (papers I, II and IX).

Many hormones are known to change the tissue levels of cyclic AMP (cAMP) and this nucleotide has been proposed as an intracellular mediator of hormonal effects (Robison, Butcher and Sutherland 1971). Amer (1970) found that CCK stimulated phosphodiesterase (PDE) prepared from the gallbladder. PDE is the cAMP inactivating enzyme and a stimulation of PDE should result in decreased tissue levels of cAMP. Andersson et al. (K.E. Andersson, R. Andersson and Hedner 1972) produced direct evidence that CCK decreased the cAMP level in the gallbladder tissue and confirmed the stimulant effect of CCK on the gallbladder PDE. These authors used the C-terminal octapeptide of CCK (C8-CCK) in their study on cholecystokinetic effects on the gallbladder. This peptide is known to produce the same mechanical and secretory effects as the extracted hormone (Mutt and Jorpes 1968, Rubin, Engel, Drungis, Dzelzkalus, Grigas, Waugh and Yiacas 1969, Hedner 1970 and paper IX).

C8-CCK was also used in studies of metabolic and mechanical effects on isolated sphincter of Oddi preparations (paper VIII). C8-CCK relaxed the sphincter as did CCK. In contrast to the finding in the gallbladder C8-CCK was seen to increase the tissue concentration of cAMP in the sphincter. The increase of the cAMP content preceded the relaxation and was probably brought about by stimulant effects of C8-CCK on adenylcyclase, the enzyme responsible for the formation of cAMP. PDE in the sphincter muscle showed increasing activity during contact with C8-CCK.

Other agents known to influence the formation or breakdown of cAMP produced the same mechanical effects in the sphincter of Oddi (papers I, II and VIII) as they did in the gallbladder (Amer 1969, paper VI and K.E. Andersson, R. Andersson and Hedner 1972). These agents included adenylcyclase stimulating (β -receptor stimulants and glucagon), PDE-activating (imidazole) and PDE-inhibiting (theophylline and papaverine) drugs (Table 1). According to the proposed mechanism of action of these compounds (for

review see Robison, Butcher and Sutherland 1971), a relaxation of biliary smooth muscle is associated with an increased intracellular cAMP concentration, while contraction is associated with decreased cAMP content in the tissue. Relaxation was accordingly also obtained when cAMP was added to the gallbladder (K.E. Andersson, R. Andersson and Hedner 1972) and sphincter of Oddi preparations (paper VIII).

Table 1

	Tension		cAMP	
	GB	SO	GB	SO
β -receptor stimulants	↓	↓	[↑]	[↑]
Glucagon	↓	↓	[↑]	[↑]
Theophylline	↓	↓	[↑]	[↑]
Papaverine	↓	↓	[↑]	[↑]
Imidazol	↑	↑	[↓]	[↓]
C8-CCK	↑	↓	↓	↑
PGE ₂	↑	↓	↓ ¹	↑ ¹
cAMP	↓	↓		

Table 1

This table summarizes metabolic effects (changes in cAMP content) and mechanical effects (tension changes) of drugs on the gallbladder (GB) and the sphincter of Oddi (SO). The arrows directed upwards represent positive changes: increases and stimulant effects. Downward directed arrows represent the opposite. Arrows within brackets are postulated effects that have not been determined.

1 Unpublished observations by K.E. Andersson, R. Andersson, P. Hedner and C.G.A. Persson.

The effects on the cAMP level induced by C8-CCK and the effects produced by cAMP and the various agents known to influence the formation and breakdown of cAMP, are consonant with the idea that tissue levels of cAMP and the mechanical activity of biliary smooth muscle can be causally related. It is generally accepted that the level of free myoplasmic calcium ions regulates the contractile activity of a muscle (Sandow 1965, Ebashi and Endo 1968, Sandow 1970). R. Andersson and K. Nilsson (1972) found that cAMP stimulated the activity of a calcium-accumulating microsomal

fraction of intestinal smooth muscle. This observation suggests a mechanism by which the intracellular concentration of free calcium ions can be influenced (R. Andersson, Lundholm, Møhme-Lundholm and K. Nilsson 1972). Therefore in the gallbladder and in the sphincter of Oddi, the intracellular content of cAMP may be involved in a regulation of free calcium ions. Changes in the cAMP-level may then be of relevance for the initiation of a mechanical response. The importance of a cAMP-dependent Ca^{2+} accumulating mechanism for the contractile activity of various smooth muscles has not been settled. For example, results obtained in uterine muscle are not in favour of a general involvement of cAMP in the regulation of contractile changes in smooth muscle (Batra and Daniel 1971 a, Batra and Daniel 1971 b, Polacek, Bolan and Daniel 1971, Polacek and Daniel 1971).

Nervous regulation of biliary motility

A. Parasympathetic effects

The biliary tract is supplied with parasympathetic nerves through the vagus (Hirt 1934, Rothman 1965). Vagal denervation of the upper abdominal viscera is a common surgical procedure and the role of the vagus in biliary dynamics has received special attention (Pallin and Skoglund 1961, Beneventano, Rosen and Schein 1969, Liedberg 1969, Williams and Huang 1969, Amdrup and Griffith 1970, Fagerberg, Grevsten, Johansson and Krause 1970, Schein and Gliedman 1970, Loeweneck 1971). However, it has not been established whether the vagus is involved in the normal function of biliary motility nor whether vagotomy is causally related to any biliary disease (Hallenbeck 1967, Bouchier 1970).

Acetylcholine contracts both the gallbladder and the sphincter of Oddi (Hallenbeck 1967, papers II, VI and IX). This finding and the results of nerve stimulation suggest that the vagus mainly provides the biliary tract with motor fibres (Ivy 1934, Pallin and Skoglund 1961, Crema, Berté, Benzi and Frigo 1964, Pallin and Skoglund 1964). The contraction induced by vagal stimulation cannot by itself evacuate the gallbladder (Ivy 1934) but may increase the contraction of the gallbladder produced by CCK (Pallin and Skoglund 1964). Crema, Berté, Benzi and Frigo (1964) report and review constrictor responses of the choledochoduodenal junction during vagal stimulation. These observations agree with the finding that acetylcholine contracts the sphincter of Oddi (Crema, Benzi and Berté 1962, Hallenbeck 1967, and papers II and IX) *in situ* and in the isolated preparation.

B. Distribution and function of adrenoceptors in the gallbladder and the sphincter of Oddi

Ahlquist (1948) advanced the concept that adrenergic neurotransmitters and sympathomimetic agents act on special receptors (adrenoceptors), classified by him as α - and β -receptors. The differentiation of these receptors was based on the rank order of potency in various organs of 6 sympathomimetic amines. This classification has proved most useful and now highly selective stimulants and blockers of α - and β -receptors are known. The definition of α - and β -receptor effects has formed a basis for a better understanding of possible adrenergic functions in various organs.

In determining the presence of α - and β -receptors in the gallbladder and the sphincter of Oddi various adrenoceptor agonists and antagonists were

used (papers I—IX). Both the gallbladder and the sphincter of Oddi are shown to contain contraction-mediating α -receptors and relaxation-mediating β -receptors (papers I, II, VI and IX). However, the gallbladder (isolated and *in situ*) seems to have only a small population of α -receptors as the α -receptor mediated contraction in response to noradrenalin and adrenalin was weak and could only be obtained when the β -receptors had been blocked. A similar, small population of excitatory α -receptors has earlier been described for bronchial (H. Persson and Johnson 1970) and urinary bladder detrusor (Edvardsen and Setekleiv 1968) smooth muscles. In the untreated (no β -blocking agent present) isolated sphincter of Oddi, the adrenergic amines (noradrenalin and adrenalin) consistently produced contraction and increased activity (Magee 1946, Crema, Benzi and Berté 1962 and paper II). In contrast to the findings of other authors (Benzi, Berté, Crema and Frigo 1964), the present work confirmed this excitatory α -receptor function in the cat sphincter of Oddi *in situ* (papers I, VII and IX). The sphincter of Oddi thereby resembles other sphincters along the gastrointestinal tract (Munro 1951, Gazet and Jarret 1964, Christensen and Daniel 1966, Parks, Fishlock, Cameron and May 1969 and Kerremans and Penninckx 1970) where excitatory responses to α -receptor stimulation have been seen. The major part of the intestine otherwise contains α - and β -receptors which both mediate relaxation (Ahlquist and Levy 1959). This synergism between adrenoceptor functions seems to apply also to the duodenal tissue surrounding the sphincter of Oddi (papers II, VII and IX). Most intestinal muscle is thus, by the α -receptor mediated relaxation, distinguished from biliary smooth muscle where contraction-mediating α -receptors were found (papers II and VI).

There is pharmacological evidence (Paton and Vizi 1969, Kosterlitz, Lydon and Watt 1970), supported by morphological findings (Norberg 1964), that an inhibitory α -receptor is situated on cholinergic ganglia or axons in the intestine. However, intestinal smooth muscle seems also to contain inhibitory α -receptors (Bowman and Hall 1970). It is possible that adrenoceptors are localized to neuronal structures as well as to smooth muscle cells in the biliary tract. The present results, however, did not suggest the presence of inhibitory α -receptors in the gallbladder or the sphincter of Oddi (papers II, VI and IX).

In terms of distribution and function of adrenoceptors, it may be of interest to compare the extrahepatic biliary tract with the urinary bladder. Both organs have a storage function and intermittently expel their contents. In the urinary bladder the detrusor muscle was shown to contain mainly β -receptors which were inhibitory in function; noradrenalin and adrenalin relaxed the detrusor (Edvardsen and Setekleiv 1968). This corresponded

well to the findings with gallbladder muscle (paper VI). The urinary bladder base, similarly to the sphincter of Oddi, responded with contraction to the adrenergic amines. Based on this finding a sphincter function was proposed for the bladder base (Edvardsen and Setekleiv 1968). Various parts of the gallbladder, however, showed a uniform adrenoceptor pattern (VI). This finding lends no support to a sphincter function in the gallbladder collum (cf. Rothman 1965). Such a function of the sphincter of Oddi is then stressed by the similarity to the urinary bladder base, concerning the adrenergic response, as well as to other sphincters along the gastrointestinal tract (discussed above).

In view of the therapeutic interest in pharmacological agents that relax biliary smooth muscle, the sensitivity of gallbladder and sphincter of Oddi preparations to a selective β -receptor stimulating drug, terbutaline (Bergman, H. Persson and Wetterlin 1969) was studied.

Lands et al. (Lands and Brown 1964, Lands, Groblewski and Brown 1966, Lands, Arnold, McAuliff, Luduena and Brown 1967, Lands, Luduena and Buzzo 1967) studied the effect of β -receptor stimulating agents on various organs. Based on potency rank order differences (as in Ahlquist's study 1948) between the compounds in different organs, they suggested that different β -receptors exist, β_1 - (e.g. in the heart) and β_2 - (e.g. in the lung). H. Persson and co-workers (H. Persson and Johnson 1970, H. Persson and Olsson 1970) have substantiated the presence of different β -receptors in investigations with different muscles (heart and lung) from the same animal under identical experimental conditions. Further support for the subdivision of β -receptors is given by recently characterized selective (on lung) β_2 -receptor agonists such as terbutaline (H. Persson and Johnson 1970) and selective (on heart) β_1 -receptor antagonists such as practolol (Levy and Wilkenfield 1969). With terbutaline it has been confirmed that β_2 -receptors are also present in the vascular system (H. Persson and Olsson 1970) in skeletal muscle (Olsson 1972) and in the uterus (Olsson and C.G.A. Persson 1971).

The potency of terbutaline in relaxing biliary smooth muscle was compared with that of another nonselective β -receptor agonist, isoprenaline. Terbutaline was shown to relax the gallbladder and sphincter of Oddi preparations to the same extent as isoprenaline (papers II and VI). Terbutaline thus seemed to be a full β -receptor agonist on biliary muscles (papers II and VI). The potency ratios between terbutaline and isoprenaline obtained in gallbladder and sphincter of Oddi preparations suggested that, compared to isoprenaline, terbutaline was more active on biliary smooth muscle (papers II and VI) than on heart muscle (H. Persson and Olsson 1970). It was further demonstrated that terbutaline counteracted the spasmogenic effect

of morphine at the choledochoduodenal junction (paper VII). This effect could be reproduced in humans where it was shown that terbutaline decreased the intrabiliary pressure that had been increased by morphine (Ingemarsson, Liedberg and C.G.A. Persson 1972). Isolated human gallbladder preparations could also be relaxed by terbutaline (paper VI).

C. Effect of adrenergic nerve stimulation on the gallbladder and on the sphincter of Oddi

The splanchnic nerves are the main source of sympathetic innervation to the upper abdominal viscera in the cat (Hirt 1934). Baumgarten and Lange (1969) using the Falck-Hillarp fluorescence technique have shown that adrenergic nerves, probably containing noradrenalin, are present in the gallbladder and the sphincter of Oddi of the cat. Results obtained with tyramine, noradrenalin and adrenalin on isolated gallbladder strips taken from normal and reserpinized cats suggest that the adrenergic nerves relax the gallbladder (paper VI). Similar studies performed on the isolated sphincter of Oddi show that the sphincter is contracted when the adrenergic transmitter amine is released by tyramine (paper II). These adrenergic effects on biliary smooth muscle can be explained in terms of adrenoceptor population and functions in the gallbladder (papers VI and IX) and the sphincter of Oddi (papers I, II and IX), as discussed above.

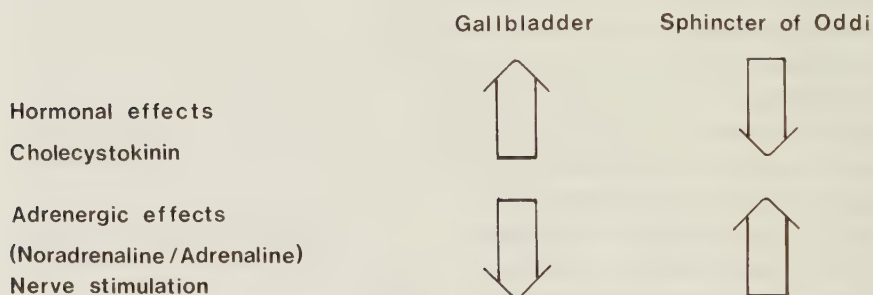
In agreement with the *in vitro* findings it was shown that stimulation of the right great adrenergic splanchnic nerves, as well as i.v. noradrenalin, mainly activated α -receptors in the sphincter of Oddi (inducing contraction) and β -receptors in the gallbladder (inducing relaxation) (paper IX). The finding that stimulation of the right splanchnic nerves relaxes the gallbladder agrees with the results obtained by Bainbridge and Dale (1905—1906) and Pallin and Skoglund (1964). It seems, however, that the gallbladder pressure has to be elevated (e.g. by CCK) before a clear-cut relaxation is regularly obtained by stimulation of the adrenergic nerve supply to the gallbladder (paper IX).

Inconsistent responses to noradrenalin, adrenalin and adrenergic nerve stimulation in the sphincter of Oddi are reported in the literature (for reviews see Ivy 1934 and Hallenbeck 1967). As mentioned under 'Experimental Approach' and discussed in paper IX, it should be of particular importance to have a method which selectively records the resistance of the sphincter of Oddi when sympathetic and sympathomimetic effects are evaluated. This reasoning seemed valid because the contiguous duodenal musculature is relaxed by adrenergic stimuli including α -receptor activation (papers II and IX); while, judging from *in vitro* and *in situ* experiments, the

sphincter of Oddi is contracted in response to such stimulation (papers I, II and IX). In paper IX it is discussed how the methods employed (e.g. higher perfusion rates through the sphincter than used in the present work) may have contributed to the difficulties experienced by other authors (Benzi, Berté, Crema and Frigo 1964, Crema, Berté, Benzi and Frigo 1964) in confirming *in situ* the contraction that is produced by the adrenergic amines in isolated sphincter of Oddi preparations (Magee 1946, Crema, Benzi and Berté 1962 and paper II).

From the studies presented in paper IX it was moreover evident that adrenergic stimuli, in relaxing the gallbladder, counteracted the CCK-induced contraction. In the sphincter of Oddi the situation was the reverse. CCK produced relaxation and adrenergic stimuli contraction. These findings showed that the effects of splanchnic adrenergic innervation counteracted hormonal effects on biliary motility (Fig. 2).

**Fig 2 Hormonal and adrenergic reciprocal contractile effects
on extra-hepatic biliary motility**



In summary: the dual adrenergic effects on the gallbladder (relaxation) and the sphincter of Oddi (contraction) are produced by the adrenergic amines (noradrenalin and adrenalin) both in isolated preparations and in *in vivo* studies; the dual effects are obtained through release by tyramine of the adrenergic transmitter in the isolated organs and also *in situ* when the adrenergic nerve supply to the biliary tract is stimulated. Finally, the dual effects are explained by the finding that, in the gallbladder, the β -adrenoceptors dominate while in the sphincter there is a more even distribution between α - and β -receptors. It is possible that the adrenergic effects are of significance during the gallbladder filling phase, promoting the entry of bile into the gallbladder.

Nature and function of the spontaneous activity of the sphincter of Oddi

It has been observed by many workers that the sphincter of Oddi exhibits spontaneous activity (Lueth 1931, Magee 1946, Bergh and Layne 1940, Crema, Benzi and Berté 1962, Wyatt 1967). In studies II, III, V and VII various patterns of spontaneous sphincter activity are shown. In the *in vitro* experiments, both tension changes in the longitudinally mounted sphincter of Oddi and resistance to flow through the sphincter (by constant rate perfusion through a cannula in the distal common bile duct) are recorded. Each change in tension, spontaneous or drug-induced, was accompanied by a corresponding pressure change. This parallelism was seen in detail because the constant rate perfusion of the sphincter provided continuous recording of the resistance to flow through the sphincter. Longitudinal tension and resistance to flow seemed to be interrelated because of the parallel recordings and because mechanical stretching of the sphincter preparation, simulating the longitudinal movements of the sphincter, did not cause changes in the resistance to flow (paper II). The spontaneous activity of the sphincter of Oddi was shown to be myogenic in origin as it was not inhibited by agents known to block nervous effects (paper II). In this respect the sphincter is comparable in behaviour to many other visceral smooth muscles (Holman 1968). *In situ* the sphincter showed a spontaneous activity that resembled the *in vitro* recordings (papers I and IX). The sphincter activity *in situ* was also affected by pharmacological agents in the same way as the isolated preparation (papers I, II and IX).

Boyden (1957a) in his study on muscle arrangements in the cat chole-
dochoduodenal junction, reported that, in the proximal part of the intra-
mural sphincter of Oddi, the pancreatic and bile ducts are separated by a
muscle septum, which according to him might selectively influence the bile
duct. This report made it also of interest to study the resistance to flow
through the pancreatic duct, by the isolated sphincter of Oddi (paper V).
Both pancreatic and bile ducts were perfused simultaneously in the longi-
tudinally mounted sphincter preparation (paper V). Perfusion pressures and
isometric tension were recorded. A close parallelism between the three re-
cordings, tension changes and changes in both perfusion pressures was
shown. This concerned both the spontaneously active sphincter and the

sphincter under the influence of drugs (paper V). This finding lends no support to a functional significance of the possibility pointed out by Boyden (1957a) that pancreatic and bile flow may be selectively affected by the sphincter. However, the results can be linked with the view that the cat sphincter of Oddi is a functional syncytium as discussed by Dewey and Barr (1968) for smooth muscle organs.

Various opinions have been expressed on the role of the sphincter of Oddi in the transport of bile into the duodenum. Watts and co-workers (Watts and Dunphy 1966, Toouli and Watts 1972) suggested that the sphincter acts as a pump activated by CCK. They performed experiments on the dog sphincter of Oddi *in situ* and *in vitro*. It may be said in this connection that Boyden in his anatomical investigations found more similarities between cat and human sphincter of Oddi than between dog and human sphincters (Boyden 1957a). Watts and Dunphy (1966) in their *in situ* study have actually illustrated a biphasic response to CCK where the effect with the longest duration is a relaxation of the sphincter. In the isolated sphincter of Oddi, Toouli and Watts (1972) obtained no response to CCK in four out of nine preparations. In three, slight contractions were obtained and in one preparation CCK inhibited the spontaneous activity. It seems then, that the conception that CCK relaxes the sphincter of Oddi as substantiated by other workers (Sandblom, Voegtlin and Ivy 1935, Magee 1946, Crema, Benzi and Berté 1962, Wyatt 1967, Hallenbeck 1967, Hedner and Rorsman 1969) is better founded than the view presented by Watts and co-workers (Watts and Dunphy 1966, Toouli and Watts 1972).

In the isolated perfused sphincter of Oddi it was consistently shown that increased spontaneous activity or drug-induced activity was associated with increased resistance to flow through the sphincter (papers II and V), and CCK consistently inhibited the sphincter activity and decreased flow resistance (papers II and V). The metabolic effects of CCK (paper VIII) support this finding. When the perfusion tube was disconnected from the perfusion apparatus and held so that the perfusion pressure was kept constant, the spontaneous activity of the sphincter of Oddi was insufficient to decrease the fluid level in the tube (paper II). Thus it was shown that the sphincter activity has no propulsive function but is associated with increased resistance to flow through the sphincter.

Tetrodotoxin (TTX) was found to increase the spontaneous activity of the isolated sphincter of Oddi (paper III). TTX also seemed to potentiate the excitatory effects of acetylcholine and noradrenalin (paper III). TTX has been shown to abolish action potentials by interfering with the sodium permeability in nerve fibres (reviewed by Kao 1966). Smooth muscle re-

activity is not affected by this action and thus TTX is recommended as a convenient 'denervating' agent in experiments on isolated smooth muscle organs (Gershon 1967). The excitatory effect of TTX on the sphincter of Oddi seemed difficult to explain by actions on nerve structures (paper III). However, Wood (1972) quite recently studied excitatory electrophysiological and mechanical effects of TTX on circular intestinal muscle. He produced evidence that the stimulant effect of TTX was not directly on the muscle but involved inhibition of spontaneously active inhibitory neurones. If this was true for the effect of TTX on the sphincter of Oddi, the spontaneously active inhibitory neurones would neither be cholinergic nor adrenergic in nature because no consistent change in the sphincter activity was seen when effective doses of atropine and adrenoceptor blocking agents were added to the isolated sphincter (papers II and III). The contractile effect of TTX might then be regarded as indicating a localization of non-adrenergic inhibitory nerves to the sphincter of Oddi. There is cumulating evidence for the presence of non-adrenergic inhibitory nerves as well as of non-cholinergic excitatory nerves in the gastro-intestinal tract (reviewed by Bortoff 1972). To my knowledge no studies on the presence and function of non-adrenergic and non-cholinergic nerves in the biliary tract have been performed.

Mechanism of action of morphine at the choledochoduodenal junction

Morphine and related analgesics have important effects on biliary dynamics. They increase the intraductal pressure in the biliary tract. This effect is due to increased resistance to flow through the choledochoduodenal junction (for reviews, see Hallenbeck 1967, Daniel 1968). It is an unwanted side effect of morphine therapy. However, the morphine induced spasm has also been used for diagnostic purposes (Sørensen 1964) and it is a common procedure in the evaluation of spasmolytic effects on human biliary smooth muscle, to use morphine as a spasmogenic agent (Hallenbeck 1967, Kewenter and Kock 1971, Ingemarsson, Liedberg and C.G.A. Persson 1972). Nevertheless, it has not been shown whether the obstruction of the choledochoduodenal junction is due only to the morphine-increased tone of intestinal muscle or to pharmacological effects on the sphincter of Oddi as well (paper IV). Since isolated preparations of both duodenum and sphincter of Oddi are insensitive to morphine, no conclusion could be drawn from *in vitro* experiments concerning possible effects of morphine on the sphincter (paper IV).

It seemed worthwhile to study *in situ* the effect of morphine and the duodenal influence on the sphincter more closely (paper VIII). In these experiments there was good agreement between intensity and duration of the effect of morphine on the perfusion pressures through the sphincter of Oddi and through the venous insertions used for selective recording of duodenal activity close to the sphincter (paper VII). This finding indicated that the morphine-increased passage-pressure through the sphincter of Oddi could be due exclusively to an effect on the intestine.

The only evidence suggesting that morphine had an effect on the smooth muscle of the sphincter was obtained in studies on the combined effect of morphine plus noradrenalin. The point was to make use of the differential effect of noradrenalin on sphincter (contraction) and duodenal muscle (relaxation). A dose of noradrenalin that neutralized the morphine-increased duodenal tone was injected during the action of morphine. If under these conditions noradrenalin caused the passage pressure through the sphincter of Oddi to rise higher than it did with noradrenalin alone, this would indicate that morphine, besides its effect on the duodenum, increased the tone

in the smooth muscle of the sphincter of Oddi. As reported in paper VII this kind of evidence was obtained in favour of an excitatory effect of morphine on the sphincter of Oddi.

Some other aspects on the spasmogenic action of morphine were dealt with in paper IV. It was shown that an effective block of atropine-sensitive cholinceptors and of α -adrenoceptors was insufficient to prevent the morphine spasm. These results confirmed the findings of Crema, Benzi, Frigo and Berté (1965). However, in their study they had not included agonists to show that the block was effective. It was further shown that the effect of morphine in doses of only 1 μ g/kg or less could be counteracted by atropine (paper IV).

Crema, Benzi, Frigo and Berté (1965), working with the cat, did not record any change in sphincter resistance when they infused intra-arterially morphine doses which were even higher than those effective when given intravenously. This finding was surprising since other authors have shown that morphine regularly produces spasm of other parts of the intestine when given intraarterially (Daniel 1966, Burks and Long 1967). In contrast to the report by Crema, Benzi, Frigo and Berté (1965) morphine was shown to consistently increase the resistance to flow through the sphincter of Oddi and increase duodenal tone when given intraarterially in about 1/100 of the dose which produces an effect when given intravenously (paper IV). The dose ratio between the two routes of administration is about what can be expected, taking into account the dilution factor of the circulating blood and assuming no special concentration of the drug in choledochoduodenal tissues. This finding suggests that morphine acts by the same mechanism independent of the route of administration. It is concluded that morphine exerts its spasmogenic effect locally in the sphincter region through release of some endogenous contracting agent (paper IV).

General summary

The present work has mainly been concerned with adrenergic, cholecystokinetic and morphine-induced effects on the cat gallbladder and choledochoduodenal junction. The results obtained can be summarized as follows:

1/ Two methodological developments are of importance, a) *in vitro* and *in situ* perfusion of the sphincter of Oddi at a constant low rate resulting in continuous recording of the sphincter activity with minimum influence by the surrounding duodenum, b) constant low rate perfusion of venous insertions through the intestinal wall, providing selective and sensitive tracings of local duodenal activity.

2/ By the use of relevant adrenoceptor blocking agents, phenoxybenzamine for the gallbladder and propranolol for the sphincter of Oddi, it is demonstrated that CCK does not produce its mechanical effects on extrahepatic biliary smooth muscles by activation of α - or β -receptors.

3/ CCK is shown to increase the intracellular level of cAMP. The metabolic change precedes the mechanical effect and is suggested to be of importance for initiation of the relaxation of the sphincter by CCK. The cAMP-level is probably increased by stimulant effects of CCK on adenylyl cyclase as the enzyme PDE in the sphincter muscle cells shows increasing activity during contact with CCK.

4/ It is shown that other agents which are known to influence the formation or breakdown of cAMP produce mechanical effects on the sphincter of Oddi, in agreement with the conception that relaxation is associated with increased and contraction with decreased intracellular levels of cAMP.

5/ It is shown that CCK decreases intramural duodenal pressure close to the sphincter of Oddi.

6/ Evidence is provided that both the gallbladder and the sphincter of Oddi contain relaxation-mediating β -receptors and contraction-mediating α -receptors. In the gallbladder, where various parts of the organ were found to have a uniform adrenoceptor pattern, the β -receptors dominate while in the sphincter of Oddi there is a more even distribution of α - and β -receptors.

7/ The gallbladder and the sphincter of Oddi are found to be sensitive to a selective β_2 -receptor agonist, terbutaline, which seems to be a full agonist in relaxing these organs.

8/ It is demonstrated that the adrenergic amines have dual effects on the

gallbladder (relaxation) and the sphincter of Oddi (contraction). The dual effects are also obtained by release with tyramine of the adrenergic transmitter in the isolated organs and *in situ* when the adrenergic nerve supply to the biliary tract is stimulated. It is possible that the adrenergic effects are of significance during the gallbladder filling phase, promoting the entry of bile into the gallbladder.

9/ The spontaneous activity of the sphincter of Oddi (*in situ* and *in vitro*) is shown to be myogenic in origin. The mechanical changes in the sphincter of Oddi (longitudinal tension changes), spontaneous or drug-induced, are found to correspond in detail with changes in resistance to flow through the sphincter when perfused both through the bile and pancreatic ducts. It is shown that the sphincter activity has no propulsive function but is associated with increased resistance to flow through the sphincter. Tetrodotoxin is found to excite the isolated sphinchter of Oddi preparation.

10/ Morphine is shown to be ineffective on isolated preparations of duodenum and sphincter of Oddi. *In situ* a close parallelism is recorded between intensity and duration of action of morphine on intramural duodenal pressure close to the sphincter of Oddi and effect on resistance to flow through the sphincter. By simultaneous administration of noradrenalin and morphine data were obtained in favour of the view that morphine increases the tone of the smooth muscle of the sphincter of Oddi. In addition, it is shown that morphine is potent when given intraarterially and that effective doses of atropine and phenoxybenzamine do not block the spasmogenic effects of morphine.

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